REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-69 are in this case. Claims 1-52 were previously cancelled. Claims 53-69 have been rejected. Claims 53-54, 59-60, 63 and 65-69 have now been amended. Claim 64 has now been cancelled.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 53-69 under 35 U.S.C. § 112, second paragraph, as being incomplete for omitting essential steps. In particular, the Examiner points out that the omitted step used to transform the multicellular eukaryotic diploid parasite was omitted. The Examiners rejections are respectfully traversed.

With respect to claims 53-63 and 65-69, the Examiner points out that the phrase "group transformation method" in claim 53 is vague and renders the claim indefinite. In order to overcome the Examiner's rejection, claim 53 has now been amended to include the limitations of claim 64.

With respect to claims 65-68 the Examiner points out that the limitation "said transgene" lacks sufficient antecedent basis. In order to overcome the Examiner's rejection claim 65 has now been amended to recite "a transgene" instead of "said transgene".

In view of the above amendments, Applicant believes to have overcome the 35 U.S.C. § 112, second paragraph, rejections.

35 U.S.C. § 102 (b) Rejections

The Examiner has rejected claims 53-54, 59-60, 63-66 and 69 under 35 U.S.C. § 102(b) as being anticipated by Kim *et al.* (U.S. Pat. No. 5,643,718). The Examiner's rejections are respectfully traversed. Claims 53-54, 59-60, 63, 65-66 and 69 have now been amended. .

The Examiner points out that Kim *et al.* teach a method of stable transformation of a parasite of the genus *Taxoplasma*, such as *Taxoplasma gondii*,

by introducing into the parasite a vector containing a DNA sequence encoding a selectable marker, such as CAT, via electroporation and homologous recombination. The Examiner further points out that it was known in the art that *Taxoplasma gondii* is sensitive to some antibiotics, such as clarithromycin and pyrimethamine, and has distinguishable sexes. The Examiner asserts that claims 53-54, 60, 63-66 and 69 are anticipated by Kim *et al.*

The applicant wishes to point out that Kim *et al.* teach transformation of unicellular parasites. In sharp contrast, the present invention is of methods of transforming a differentiated developmental stage of a multicellular parasite.

As is well known in the art, a multicellular organism such as the schistosome described in the instant application comprises several distinct differentiated cell types interacting thereamongst to form a functional organism (see definition in last paragraph on page 9 of the instant application). Since multicellular organisms include several distinct cell types, they are inherently less amenable to transformation when compared to unicellular organisms which are composed of a single cell. Aside from hurdles presented by the presence of several cell types, transformation of differentiated multicellular organisms is further complicated by the fact that multicellular organisms are far more sensitive to environmental stress conditions present during transformation than unicellular organisms.

Since genetic transformation of single cells is far easier than co-transformation of several different cells, prior art transformation of multicellular organisms is typically effected by transforming individual non-differentiated cells of the organism followed by regeneration of individual cells into whole organisms. In fact, prior to filing the instant application, no successful transformation of a multicellular parasite has been reported. As is described in detail hereinbelow with respect to the U.S.C. § 103(a) rejections, the present inventors have uncovered that a specific developmental stage of a multicellular parasite is highly amenable to transformation thus providing the guidelines necessary for successful transformation of multicellular parasites.

Thus, clearly, the teaching of Kim *et al.* of transforming a unicellular parasite does not anticipate the teaching of the present invention of transforming developmental stage multicellular parasites.

The Examiner has rejected claims 53-54, 59-60, 63-66 and 69 under 35 U.S.C. § 102(b) as being anticipated by Roos *et al.* (Methods: A Comparison to methods in Enzymology 13:112-122, 1997). The Examiner's rejections are respectfully traversed. Claims 53-54, 59-60, 63, 65-66 and 69 have now been amended. .

The Examiner points out that Roos *et al.* teach a method of transforming *Taxoplasma gondii* via electroporation.

Applicant is of the opinion that since Roos *et al.* teach transforming a unicellular parasite they do not anticipate the present invention which teaches transforming developmental stage of a multicellular parasite, as argued hereinabove with respect to Kim *et al.*

The Examiner has rejected claims 53-54, 59-60, 63-66 and 69 under 35 U.S.C. § 102(b) as being anticipated by Watters *et al.* (Annals of Tropical Medicine and Parasitology 91: S63-S67, 1997). The Examiner's rejections are respectfully traversed. Claims 53-54, 59-60, 63, 65-66 and 69 have now been amended. .

The Examiner points out that Watters *et al.* teach a method of transforming *Plasmodium berghei* via electroporation.

Applicant is of the opinion that since Watters *et al.* teach transforming a unicellular parasite they do not anticipate the present invention which teaches transforming multicellular parasites, as argued hereinabove with respect to Kim *et al.*

In view of the above arguments Applicant believes to have overcome the 35 U.S.C. § 102 (b) rejections.

35 U.S.C. § 103(a) Rejections

The Examiner has rejected claims 53-69 under 35 U.S.C. § 103(a) as being unpatentable over Miller (WO 97/11191) in view of either Kim *et al.* (U.S. Pat. No. 5,643,718) or Roos *et al.* (Methods: A Comparison to methods in Enzymology 13: 112-122, 1997). The Examiner's rejections are respectfully traversed. Claims 53-54, 59-60, 63, 65-66 and 69 have now been amended.

In particular, the Examiner points out that Miller *et al.* teach a method of transforming schistosome via microinjection of transgenic DNA into stage I schistosome eggs.

Kim *et al.* teach a method for stable transformation of *Toxoplasma* species via electroporation and homologous recombination. Kim *et al.* also teach recombinant expression of an obligate intracellular parasite of the phylum *Apicomplexa.*

Roos *et al.* teach a method for stable transformation of *Toxoplasma gondii* via electroporation.

The Examiner asserts that it would have been obvious and within the scope of one ordinary skilled in the art at the time the invention was made to substitute the microinjection method as taught by Miller *et al.* with electroporation and homologous recombination as taught by Kim *et al.* or Roos *et al.* for stable transformation of animal cells and to make genetically modified animal including parasites.

The Applicant wishes to point out that although Miller *et al* describe and claim microinjection as a means of transformation, they fail to provide experimental data to support their invention. While reducing the present invention to practice, the inventors of the present invention tried to microinject schistosome eggs essentially as described by Miller *et al*, yet failed to obtain transformed schistosomes. This issue is addressed in the last paragraph of the Background section of the instant application, stating in this respect that: "PCT Application No. US96/15083 by Miller, published as WO 97/11191, teaches a method of producing schistosomes as an intermediate transgene vector for secretion of desired gene products into the bloodstream of a host. The method disclosed is, however, based solely on microinjection of schistosome eggs, whereas no experimental support for the

applicability of microinjection for genetically transforming schistosome eggs is provided. While reducing the present invention to practice, as is further detailed in the Examples section that follows, extensive efforts were made to genetically transform schistosome eggs by microinjection of genetic material therein, however, not even a single transformant was recoverable by this procedure." Apparently, Miller *et al.*, while drafting a theoretical description of transforming schistosomes failed to realize that the egg microinjection procedure is ineffective. It is therefore the Applicant opinion that Miller *et al* fail to teach one of ordinary skill in the art how to transform schistosomes.

Notwithstanding from the above, even if the method of Miller *et al.* was effective, the method of the instant application is distinct and substantially advantageous over the method of Miller *et al.*

The present invention teaches transformation of multicellular developmental stage organisms (schistosome's miracidia) via group transformation techniques (e.g., electroporation, chemical transformation, lipofection and biolistic bombardment). The miracidia are taken for transformation when still within eggs or, alternatively, after they are released from the eggs by hatching under hypotonic conditions. The selection of miracidia for transformation is based both on their position in the life cycle prior to clonal multiplication, as well as on their anatomy which favors introduction of foreign genes into germ cell (which is the term used in the art of parasites for somatic stem cells) as explained below.

The miracidium is a ciliated multicellular organism with four epidermal plates arranged in four tiers and covered with cilia and apical musculature and glandular structure suitable for penetrating the snail host. Most of the posterior third portion of the organism is filled with a cluster of germinal cells (the somatic stem cells), which are interconnected and are also connected to the surface of the organism. Germ cells (i.e., somatic stem cells) undergo multiplication shortly after the miracidium penetrates into the snail and transforms into a primary (mother) sporocyst which is the starter of asexual multiplication to form daughter sporocysts and subsequently cercariae which infect the vertebrate host and develop into adult worms. In addition, miracidia are highly acidophilic and are located in highly

perforated ova capable of allowing passage of macromolecules. These features combined are favorable for introducing nucleic acids into miracidial germ cells.

Mature eggs containing fully developed miracidia are suitable for transformation because of the anatomy of the fully developed miracidium within them as described above.

Schistosome lay immature eggs, which undergo development to fully embrionated eggs within a few days after they have been laid. Therefore, collection of mature schistosome ova for transformation needs to be done from tissues of an infected animal where eggs become trapped, such as the intestines and livers of infected mice. A large proportion of such eggs is fully embrionated. Collecting large amounts of eggs is essential for group transformation. Naked miracidia with their cytoplasmatic cover and cluster of germinal cells (i.e., somatic stem cells) which are interconnected and are also connected to the surface of the organism are also suitable targets for group transformation, because of the accessibility of foreign DNA to the germ cells (i.e., somatic stem cells). They can be obtained in large numbers by hatching of eggs collected from tissues of infected animals. See in this respect also pages 21, line 23 to page 23, line 21 of the instant application.

Thus, by transforming germ cells (i.e., somatic stem cells) just prior to clonal multiplication, one ensures passage of the transgene to all of the emerging clones, not through sex cells, so as to avoid spread of sexual progeny of transgenic worms.

Applicant further wishes to point out that both Kim *et al.* and Roos *et al.* fail to teach transformation of a multicellular stage of a parasite. In sharp distinction to unicellular organisms, multicellular organisms are far more difficult to transform, as already explained hereinabove. From the teachings of Miller *et al.*, Kim *et al.* and Roos *et al.* one cannot predict the effect of such transformation on a multicellular stage of a multicellular parasite. Applicant, through a detailed and carefully designed experimental set-up which is described in great length in the Examples section of the instant application, developed experimental conditions highly suitable for transforming a multicellular parasite via a group transformation method. Furthermore, using the experimental set-up described by the instant application, one

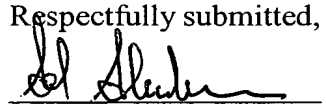
can optimize transformation conditions for a variety of multicellular organisms and developmental stages.

Therefore, it is the Applicant's opinion that the combined teachings of Miller *et al.*, Kim *et al.* and Roos *et al.* do not render obvious the invention, described and claimed in the instant application.

In view of the above arguments and claim amendments, Applicant believes to have overcome the 35 U.S.C. § 103(a) rejections.

Therefore it is respectfully submitted that claims 53-63 and 65-69 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,



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Date: January 19, 2004.

Encl.:
Three months extension fee.